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Abstract# 2547 **Poster Board#/Session: 404-IV**

OPTIMAL CYTOKINE STIMULATION FOR THE ENHANCED GENERATION OF LEUKEMIC DENDRITIC CELLS: A NOVEL STRATEGY FOR THE ADOPTIVE IMMUNOTHERAPY OF LEUKEMIA. Nicki Panoskaltsis, Todd Belanger*, Karen Rosell*, Abbe Harbol*, Jane L. Liesveld, Camille N. Abboud. *The University of Rochester Medical Center, Hematology/Oncology Unit, Rochester, NY, USA.*

Dendritic cells (DC) are a discrete population of leukocytes with potent professional antigen presenting capabilities. They are found naturally in many body tissues, as well as in circulation at different stages of maturation. Activation of DCs to express costimulatory molecules B7.1 (CD80) and B7.2 (CD86) occurs at the site of tissue inflammation, infection or recognition of non-self antigens through various cytokine and chemokine signals. It has been noted that patients with malignancy have a better outcome when increased numbers of DCs are found in malignant tissues. Therefore, their use in the immunotherapy of malignancy has become paramount in the search for alternatives to chemotherapy in resistant disease. Human acute and chronic myelogenous leukemia (AML and CML respectively) samples have been grown in culture to generate functional DCs derived from the malignant clone. Most of these reports, however, have used complex culture techniques and a 14-21 day culture period. In this work, we report the generation of DCs from different samples of human AML, CML and acute lymphocytic leukemia (ALL). Bone marrow or peripheral blood was obtained from patients with newly diagnosed or relapsed AML, CML, or ALL and either used fresh or later after cryopreservation for in vitro experiments. Mononuclear cells were isolated and cultured in RPMI with 10% FBS or 1% autologous serum depending on availability. Leukemic cells were cultured for 1, 3, and 5 days in different conditions: no growth factor, stem cell factor (SCF), GM-CSF + IL-4 + SCF, GM-CSF + TNFα + SCF, IL-3 + SCF, IL-3 + IL-7 + SCF, IL-7 + SCF, GM-CSF + Flt-3L + SCF, Flt-3L + SCF, GM-CSF + Flt-3L, and Flt-3L alone. TNF-α was added one day prior to harvest of all cultures. Cells cultured in each condition were analyzed by flow at day 0, day 1, day 3, and day 5 of culture for expression of CD40, CD1a, CD80, CD86, CD83, CD4, CD11c, CMRF-44, and IL-3Rα. In addition, cells were analyzed for DC morphology by phase-contrast microscopy and Wright's-Giemsa staining of cytospin specimens. Furthermore, FISH analysis of appropriate samples and mixed lymphocyte reactions (MLR) were performed to determine DC derivation from the leukemic clone and the ability of these cells to present antigen. Although total cell output was not increased in any of these specified conditions, functional leukemic DCs were produced within 1-3 days of culture. More specifically, cytokine combinations utilizing Flt-3L and SCF coupled with terminal TNF-α induced DC maturation optimally. Based on these results, we conclude that leukemic samples can be manipulated to generate functional DCs from the abnormal clone itself utilizing a short generation time. The ability to manipulate leukemic blasts into DCs represents a novel therapeutic strategy for the adoptive immunotherapy of leukemia.

Abstract# 2548 **Poster Board#/Session: 405-IV**

LYMPHOBLASTIC LEUKEMIA CELLS EXPRESS CXCR-4 AND MIGRATE THROUGH ENDOTHELIUM IN RESPONSE TO SDF-1: IMPLICATIONS FOR LEUKEMIA CELL VACCINATION. Angelo A. Cardoso*, J. Pedro Veiga*, Paolo Ghia, Hernani M. Afonso*, Lee M. Nadler. *Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.*

Although highly efficacious in children, treatment of acute lymphoblastic leukemia (ALL) is complicated by long-term toxicities. Strategies that might provide equivalent or greater efficacy while lessening toxicity are critical to improve the therapeutic index. One such novel strategy is the generation of anti-ALL specific immunity. We have previously shown that B-precursor ALL cells can be modified by CD40-crosslinking to become efficient antigen-presenting cells (APC) and that autologous anti-leukemia cytotoxic T cells (CTL) can be generated from the bone marrow (BM) of these patients. If these CD40-stimulated ALL cells are to be effective vaccines, they must be able to migrate and home to the sites where anti-leukemia specific T cells can be found, i.e. the BM. To determine whether ALL cells could be attracted to disease sites, we undertook an extensive analysis of the profile of chemokine receptor expression on ALL cells. We observed that both primary and CD40-stimulated leukemia cells (n = 25 patients) express significant levels of the chemokine receptor CXCR-4. This chemokine receptor, which has been shown to function as a coreceptor for the entry of HIV-1 into T cells, is the specific receptor for the chemokine SDF-1. CXCR-4 expression was confirmed at both mRNA and protein level. To assess the functional implications of CXCR-4 expression, the response of leukemia cells to SDF1 was tested using both calcium flux and a transendothelial cell migration assays. In 3 of 6 patients tested, leukemia cells respond to SDF-1 by mobilization of intracellular calcium. More importantly, in all cases tested (n = 10) both primary and CD40-stimulated leukemia cells migrate through endothelium in response to recombinant SDF-1, but not to other chemokines such as MIP-1α, RANTES, TARC, or MDC. This migration was strongly inhibited by the addition of an anti-CXCR-4 antibody. SDF-1 had no effect on leukemia cell survival or their capacity to function as APC. We then attempted to determine whether autologous BM stroma derived from leukemia patients' BM aspirates express SDF-1. In all cases tested (n = 6), BM stroma from ALL patients express both SDF-1α and SDF-1β. More importantly, supernatants from these BM stromal cultures stimulate transendothelial migration of CD40-stimulated ALL cells, which is also inhibited by an anti-CXCR-4 antibody. Taken together, these studies show that CD40-stimulated, APC-competent ALL cells express the chemokine receptor CXCR-4 and respond to stimulation by its specific ligand SDF-1. Moreover, the leukemia-permissive stromal microenvironment produces functional SDF-1. In light of our previous studies showing that anti-ALL specific CTL precursors exist in the bone marrow and that CD40-stimulated ALL cells can stimulate CTL generation and expansion, the demonstration that ALL cells can migrate through BM endothelium in response to a chemokine produced by their microenvironment, provides an additional argument that vaccination strategies for the treatment of ALL may be successful.

Abstract# 2549 **Poster Board#/Session:**

INCREASED SENSITIVITY OF ACUTE MYELOID LEUKEMIA TO LOVASTATIN INDUCED APOPTOSIS: A POTENTIAL THERAPEUTIC APPROACH. J. Dimitroulakos*, D. Nohynek*, K.L. Backway*, D.W. Ye H. Yeger*, M.H. Freedman, M.D. Minden, L.Z. Penn*. *Ontario Cancer I Princess Margaret Hospital, Toronto, Ontario; The Hospital for Sick Ch Toronto, Ontario; University of Toronto, Ontario, Canada.*

We recently demonstrated that HMG-CoA reductase, the rate limiting of de novo cholesterol synthesis, was a potential mediator of the biological of retinoic acid on human neuroblastoma cells. The HMG-CoA reductase in lovastatin, used extensively in the treatment of hypercholesteremia, ind potent apoptotic response in human neuroblastoma cells. This apoptotic re was triggered at lower concentrations and occurred more rapidly than in previously reported in other tumor derived cell lines, including breast and p carcinomas. Due to the increased sensitivity of neuroblastoma cells to lov induced apoptosis, we examined the effect of this agent on a variety of tumo including leukemic cell lines and primary patient samples. Based on a va cytotoxicity and apoptosis assays, the six acute lymphocytic leukemia ce tested displayed a weak apoptotic response to lovastatin. In contrast, hower acute myeloid leukemic cell lines (6/7) and the majority of their prima cultures (13/22) tested were extremely sensitive to lovastatin-induced apo similar to the neuroblastoma cell response. Of significance, in the acute m leukemia, but not the acute lymphocytic leukemia cell lines, lovastatin-in cytotoxicity was pronounced even at the physiological relevant concentrati this agent. Therefore, our study supports the evaluation of HMG-CoA red inhibitors as a therapeutic approach in the treatment of acute myeloid leuke

Abstract# 2550 **Poster Board#/Session:**

HMG-CoA REDUCTASE AS A TARGET FOR THERAPY IN LEUKA L.J. Woodgate*, E.J. Walker*, A.F. Gilkes*, V. Walsh*, M.C. Sweeney Mills, A.K. Burnett. *LRF Differentiation Unit, Department of Haema University of Wales College of Medicine, Cardiff, Wales, UK.*

One approach to the treatment of leukaemia is to persuade the leukaemi to complete the maturation process, which will include activation mechanism of cell death. The model for this is the use of retinoic acid in promyelocytic leukaemia. Although the response may be transient. To und the cellular and molecular events associated with retinoic sensitivity we utilised *in vitro* cell line models of *ATRA* induced differentiation. Fro comparison of the mRNA expression profiles (differential display) of two cell lines, one which is ATRA sensitive (HL60-AS) and one which responsive to this agent (HL60-AR), we identified HMG-CoA reductase (H CoA-R) as being highly expressed in myeloid cell lines that are less respons resistant to differentiation by *ATRA*. Elevated expression of this mRNA was in other *ATRA* resistant myeloid cell lines. Investigation of expression of H CoA-R in 72 primary AML samples found high levels of HMG-CoA-R excep those in the M3 FAB class (22%) which respond to *ATRA*. HMG-CoA-R the limiting enzyme in the mevalonate pathway is essential in cholesterol biosyn and farnesylation of RAS protein. The obvious role of HMG-CoA-R in proliferation makes it a target for therapy. Inhibition of HMG-CoA-R is kno have an anti-proliferative effect. Inhibitors of HMG-CoA-R include the lowering agents lovastatin and simvastatin, which inhibit at the protein *ATRA* inhibits mRNA transcription of HMG-CoA-R. Therefore there appears a rationale for using a combined therapeutic approach to the reduction of H CoA-R with the aim of increasing *ATRA* sensitivity and cell dea differentiation. Treatment of *ATRA* resistant myeloid cell lines shows an enh differentiation response with the combined use of *ATRA* and lovastatin as op to *ATRA* alone. Measured by an increase in the expression of the ATRA ind CD38 cell surface marker, the production of formazin deposits and an increa the frequency of cells that phagocytose complement coated yeast ce correlation has been identified between *ATRA* sensitivity and HMG-CoA-R in cell lines and primary material. Thereby providing a rationale for com *ATRA* and lovastatin treatment in the clinical setting.

Abstract# 2551 **Poster Board#/Session:**

IDENTIFICATION OF A SERUM-DERIVED DIFFERENTIATION-IN ING ACTIVITY AS THE COPPER-BINDING PROTEIN CERULOPLA T. Peled*, A.J. Treves*, E.A. Rachmilewitz, E. Fibach. *Dept. of Hem Hadassah University Hospital, Jerusalem, Israel.*

We found that normal serum, which sustains the growth and viability of in culture, contains a potent differentiation-inducing activity. This activity i evident when normal human or bovine sera were fractionated by either extr with organic solvents or by anion-exchange chromatography. The activity